On Mitosis in Proliferating Epithelium.

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The object of the present investigation is to determine the character of the mitosis occurring in cutaneous epithelium which has been artificially stimulated to proliferate freely. In the epithelial proliferation of carcinoma, in addition to somatic mitosis, a synaptic mitosis is also met with.* In attempting to decide upon the significance to be attached to this latter phenomenon it is necessary to ascertain whether reduction mitosis is met with exclusively in cancerous proliferation of the surface epithelium, or whether it is also encountered in non-cancerous proliferation. If it is present in the latter case, then synaptic mitosis cannot be regarded as exclusively related to epithelioma, or, for that matter, to sexual reproductive tissue; if, however, only somatic mitoses occur in non-cancerous epithelial proliferation, then the possibility is present that the altered biological characters presented by the epithelial cells of malignant growths may be in some way directly connected with the reduction taking place in the number of chromosomes.

The abundant epithelial proliferation occurring in many cutaneous diseases, *e.g.*, psoriasis, lichen planus, and dry forms of eczema, is unsuitable for this investigation, since the amount of material available from such sources is too small and the supply too inconstant to fulfil the requirements of research. Recourse was therefore had to epithelium, which had been made to proliferate freely by the use of scharlach R,† dissolved in olive oil, the injection of which beneath the skin of the rabbit's ear causes extreme and, it may, irregular hypertrophy of the Malpighian layer.‡ It was further found of advantage in obtaining an abundance of division figures to implant pieces of such proliferating epithelium under the skin of the rabbit. Using the material obtained in these two ways the present investigation was carried out. The effect of scharlach R, it may be observed, is to cause marked increase of the prickle layer, both of the surface epithelium and of the hair follicles. This increase is at first fairly regular, but later, when considerable in degree,

* Farmer, Moore, and Walker, 'Roy. Soc. Proc.,' B, vol. 77, p. 226.

† Scharlach R is azo-orthotoluol-azo-β-naphthol : $C_7H_7N = NC_7H_6N = NC_{10}H_6OH$.

‡ B. Fischer, 'Münch. Med. Wochenschr.,' 1906, 53. Jahrg., S. 2041.

becomes irregular, and ingrowth of epithelium occurs, comparable to, though considerably less extensive than, that occurring in squamous epithelioma. If the proliferation be set up in scar tissue, the resemblance to the latter condition is striking. With the disappearance of the scharlach R the process comes to an end and involution occurs, though the skin appendages may be more or less destroyed. The character of the epithelial proliferation is, therefore, intermediate between that of such affections as psoriasis and lichen planus, in which epithelial hypertrophy without downgrowth takes place, and epithelioma, in which downgrowth of epithelium is the characteristic feature, and is the means by which neighbouring tissues are invaded and extension of the growth occurs.

When marked proliferation of the epithelium of the rabbit's ear has been brought about under the conditions above described, sections hardened in Flemming's solution and stained by Heidenhain's iron alum hæmatoxylin method show numerous mitoses, amounting occasionally to as many as five mitoses to every hundred cells encountered. The mitotic cells are of large size, being 10 μ to 16 μ in diameter. In most cases the individual chromosomes can be recognised to be more or less curved rods of varying length, too closely-packed to be counted. In a relatively very small number of the mitotic figures, however, it is possible to enumerate the chromosomes, and in this way to determine the forms of mitosis present.

The form most commonly met with appears to be the ordinary somatic type shown in fig. 1,* which presents no essential points of difference from the corresponding type in the testis of the rabbit, taken as a standard,

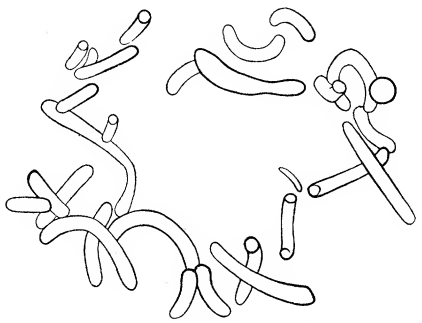

Fig. 1.—Normal Somatic Mitosis in Proliferating Epithelium of Rabbit's Ear.

* The mitoses exhibited in this and the succeeding figures, all of which have the same magnification (*cp.* scale in fig. 5), were drawn to scale in plan and elevation, and were then modelled. The illustrations were prepared from the models. The use of models was adopted in order to permit a simultaneous view of all the chromosomes present, in their natural relation, instead of the succession of views at different levels afforded by the microscope.

shown in fig. 2. In this form the number of the individual chromosomes which could be distinguished ranged between 28 and 32, while in the testis, taken for comparison, the number of chromosomes which could be recognised

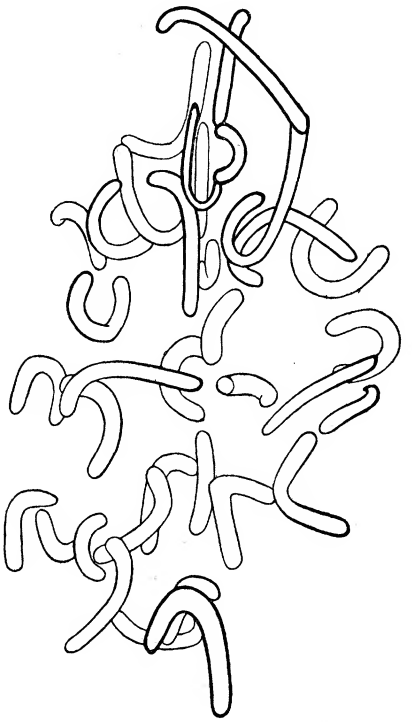

Fig. 2.—Normal Somatic Mitosis in Testis of Rabbit.

lay between 28 and 36. In the somatic mitoses of the ear of the rabbit, after injection of scharlach R, the chromosomes appear larger and coarser than in the testis, but are otherwise of the same type.

In addition to the above, another type of mitosis, shown in fig. 3, is met with less frequently than in the former. Here the chromosomes, instead of being curved rods, mostly assume the form of large spheroidal or ellipsoidal masses, taking the stain chiefly in their peripheral layer and to a less extent centrally; some of the chromosomes, however, form short rods, straight or curved, occasionally swollen at the extremities; again, some of the chromosomes form long rods (two such are shown in fig. 3, one thick, one thin). All these chromosomes are arranged in pairs, with or without connecting threads of chromatin, in the same manner as the so-called gemini of the first maiotic division of the testis (fig. 4), with the exception of the two last mentioned, which are evidently equivalent to pairs, though

no division or constriction can be recognised. It will be seen that the chromosomes in figs. 3 and 4 are on the equator of the spindle. In fig. 3

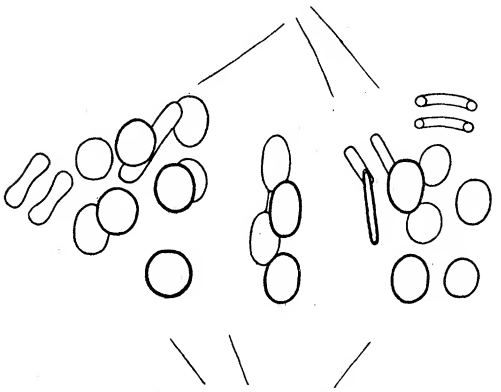

FIG. 3.—Reduction Mitosis in Proliferating Epithelium of Rabbit's Ear.

the number of pairs is 14; in other mitotic forms of the same type it was found to vary from 14 to 18. A glance at the heterotype mitosis from the testis of the rabbit (fig. 4), in which the number of gemini or pairs of chromosomes which could be counted was found to vary from 14 to 18, shows the essential morphological identity of the synaptic form occurring in the proliferating epithelium of the rabbit's ear (fig. 3) with the first maiotic mitosis in the testis of the same animal (fig. 4). In both forms the

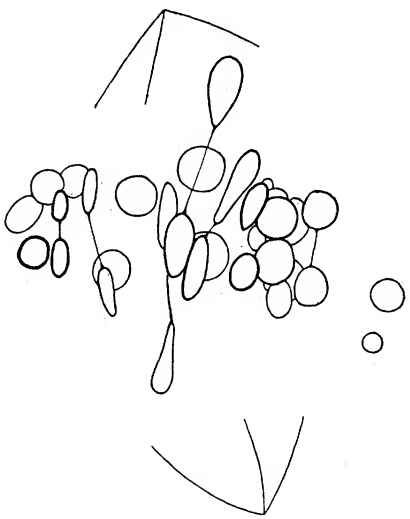

FIG. 4.—Heterotype Mitosis in Testis of Rabbit.

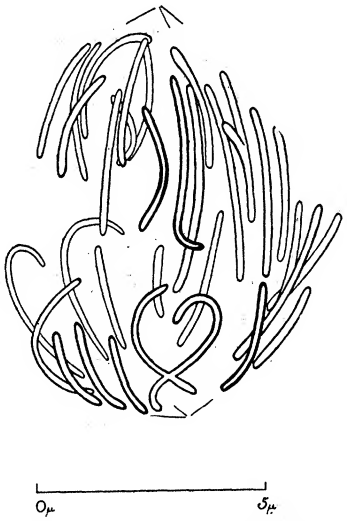

FIG. 5.—Post-synaptic Mitosis in Proliferating Epithelium of Rabbit's Ear.

chromosomes are for the most part large spheroidal or ellipsoidal masses, minor differences being recognisable in the smaller gemini.

In addition to the above type, another less frequent form of mitosis was encountered. This is exhibited in the dyaster shown in fig. 5. Here the chromosomes, which form elongated narrow rods, curved or bent, are also reduced in number, varying from 16 to 18 in each aster. This form may represent a post-synaptic division. If this is so, it is impossible to determine whether it is the first or a subsequent post-synaptic division, since there is no means of identifying its particular generation.

Spireme forms are fairly common. In these the chromatin granules are not unfrequently abundant, but well-defined double beading of the spireme thread has not so far been observed. It may be noted, however, that in the testis of the rabbit double beading could not be clearly exhibited. Late prophases, in which different permanent forms of the chromosomes composing the gemini, similar to those described by Moore and Arnold,* were recognisable, have not been met with, nor have masses of archoplasm or archoplasmic vesicles been up to the present encountered in proliferating epithelium of the rabbit's ear.

No suggestion can be offered, from the observations made in the course of this investigation, as to the significance of the reduction division which takes place. Nor has it been possible to ascertain the further history of the reduced cells, and, in particular, to determine whether division with the reduced number of chromosomes may be repeated for more than one generation.

It may be observed that the number of chromosomes counted has not been constant, but has varied from 28 to 36 in somatic mitoses, and from 14 to 18 in the reduction mitoses. The difficulty of accurately ascertaining the number present under the conditions obtaining in stained sections viewed under high magnification may in part account for the variation met with, particularly in the former case. It is, however, not at all clear that this explanation is completely satisfactory; on the contrary, the constancy with which variation is met with suggests, particularly when reduction occurs, and enumeration of chromosomes becomes in consequence much less difficult, that the number is not absolutely fixed, but exhibits a certain degree of variation.

Considerable variation was exhibited in different experiments in respect of the number of division figures encountered. As a rule, the number was greatest when the epithelial proliferation was most marked. An extreme degree of mitosis was observed at the end of a week in some of the implanted

* 'Roy. Soc. Proc.,' B, 1906, vol. 77, p. 563; also Moore and Embleton, *ibid.*, p. 555.

portions of skin. The reduction mitoses were met with in those cases in which proliferation was considerable, but owing to the very large number of division figures which are not sufficiently well defined to enable their character to be determined, it is not possible to give statistics of the relative frequency of the somatic and reduction mitoses occurring at different periods in the proliferating epithelium.

Summary.

The preceding observations may be briefly summarised as follows:—

1. In epithelial proliferation brought about by scharlach R, both normal somatic and reduced mitoses occur. This statement applies to epithelium proliferating *in situ*, and also to the same implanted under the skin.

2. In the reduction mitoses the number of chromosomes which could be counted varied from 14 to 18. In the somatic form the number counted varied from 28 to 36.

3. Reduction mitoses could be recognised less frequently than somatic mitoses.

4. Post-reduction mitoses were met with.

5. The character of the mitoses occurring was not definitely altered by implantation under the skin.